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Postinfarction heart failure in rats is associated with upregulation of GLUT-1 and downregulation of genes of fatty acid metabolism

Nathalie Rosenblatt-Velin, Christophe Montessuit, Irène Papageorgiou, Jérôme Terrand, René Lerch*

Cardiology Center, University Hospital, 24, rue Micheli-du-Crest CH-1211 Geneva 14, Switzerland

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Abstract

Objectives: Increasing evidence suggests that left ventricular remodeling is associated with a shift from fatty acid to glucose metabolism for energy production. The aim of this study was to determine whether left ventricular remodeling with and without late-onset heart failure after myocardial infarction is associated with regional changes in the expression of regulatory proteins of glucose or fatty acid metabolism. **Methods:** Myocardial infarction was induced in rats by ligation of the left anterior descending coronary artery (LAD). In infarcted and sham-operated hearts the peri-infarction region (5-mm zone surrounding the region at risk), the interventricular septum and the right ventricular free wall were separated for analysis. **Results:** At 8 and 20 weeks after LAD ligation, the peri-infarction region and the septum exhibited marked re-expression of atrial natriuretic factor [+252±37 and +1093±279%, respectively, in the septum ($P<0.05$)] and of α-smooth muscle actin [+34±10 and +43±14%, respectively, in the septum ($P<0.05$)]. At 8 weeks, when left ventricular hypertrophy was present without signs of heart failure, myocardial mRNA expression of glucose transporters (GLUT-1 and GLUT-4) was not altered, whereas mRNA expression of medium-chain acyl-CoA dehydrogenase (MCAD) was significantly reduced in the peri-infarction region (−25±7%; $P<0.05$). In hearts exhibiting heart failure 20 weeks after infarct-induction there was a change in all three ventricular regions of both mRNA and protein content of GLUT-1 [+72±28 and +121±15%, respectively, in the peri-infarction region ($P<0.05$)] and MCAD [−29±9 and −56±4%, respectively, in the peri-infarction region ($P<0.05$)]. **Conclusion:** In rats with large myocardial infarction, progression from compensated remodeling to overt heart failure is associated with upregulation of GLUT-1 and downregulation of MCAD in both the peri-infarction region and the septum.

Keywords: Energy metabolism; Gene expression; Heart failure; Hypertrophy; Infarction; Remodeling

1. Introduction

Shortly after birth, the substrate pattern of energy metabolism changes from glucose to fatty acids, which is the primary source of adenosine triphosphate production in adult myocardium [1]. This postnatal change in substrate use is associated with upregulation of several regulatory proteins involved in glucose or fatty acid metabolism [2–4].

A number of studies indicate that left ventricular remodeling in response to pressure overload [5–7], volume overload [8] or large myocardial infarction [9] is associated with an increase of both glycolysis and glucose oxidation and a decrease of fatty acid oxidation. Recent evidence suggests that metabolic changes are associated with altered expression of the regulatory proteins of metabolism, compatible with regression to a fetal expression pattern of genes of metabolic regulation, similar to observations concerning other genes [10]. For example, in rats with spontaneous hypertension, myocardial mRNA content of the 'adult' isoform of glucose transporters, GLUT-4 and of the medium-chain acyl-CoA dehydrogenase (MCAD) are decreased [11,12]. MCAD is a key enzyme involved in β-oxidation of medium-chain fatty acids (C_4–C_{12}). Since medium-chain fatty acids originate not only from exogenous supply but also from β-oxidation

*Corresponding author. Tel.: +41-22-372-7202; fax: +41-22-372-7229.

E-mail address: rene.lerch@hcuge.ch (R. Lerch).

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of long-chain and very long-chain fatty acids, the enzyme is also involved in the control of oxidation of long-chain and very long-chain fatty acids.

Myocardial infarction is a frequent cause of left ventricular remodeling and failure. However, the cellular and molecular features of postinfarction remodeling may substantially differ from pure pressure or volume overload-induced hypertrophy by factors including inhomogenous distribution of both myocardial wall stress and hypertrophy. In previous work, we showed in a rat model of chronic myocardial infarction that increased glucose oxidation was associated with reduced expression of MCAD in left ventricular myocardium 8 weeks after infarct induction [9]. However, both the expression pattern of regulatory genes of metabolism and the regional distribution of observed changes are likely to evolve during progressive cavity dilatation [13]. The precise knowledges of the regional gene alterations could have clinical implications. Therefore, this rat model of chronic myocardial infarction was studied for 20 weeks in order to assess (1) regional differences in the expression of regulatory proteins of metabolism and (2) the changes occurring during transition from compensated remodeling to overt heart failure.

In this study, we observed that the alteration of expression of metabolic genes was different among both the regions of the infarcted hearts and the time-points after infarct induction. Furthermore, the mRNA and protein alterations of the metabolic genes studied were enhanced by the presence of heart failure compared to the alterations observed in compensated hypertrophy. Finally, our results suggest that GLUT-1 expression may provide a molecular marker for the development of heart failure.

2. Methods

2.1. Experimental myocardial infarction

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Coronary ligation or sham-operations were carried out in male OFA rats (IFFA Credo, L'Arbresle, France) as described previously [9]. Sham-operated rats were age-matched with infarcted rats. Mortality of sham-operated rats during the 20 weeks observation period was extremely low (0.5%). In contrast, mortality after induction of large myocardial infarction was high due to a perioperative or immediately postoperative death rate of 65% within the first 3 days.

2.2. Experimental groups

Rats were fasted 24 h prior to sacrifice for excision of the heart for extraction of RNA and proteins.

For isolation of RNA and proteins, rats were reanesthetized with sodium pentobarbital (60 mg/kg, i.p.; Nembutal, Abbott Laboratories, Chicago, IL, USA) 24 h, 8 weeks or 20 weeks after surgery and weighed. Heart failure was identified by the criteria of Feldman et al. [14]. Rats exhibiting the following five criteria were considered to present heart failure: lethargy, pericardial effusion, pleural effusion, ascites and left atrial dilation. The time-points for measurement of gene expression were selected based on observations of a previous study [9] and pilot experiments indicating that signs of heart failure were absent after 8 weeks, but present in approximately 50% of the rats after 20 weeks. Later than 20 weeks, mortality of rats with infarction increased substantially.

After sacrifice, the heart was quickly removed. The infarcted zone, a 5-mm broad region surrounding infarction (peri-infarction region), the interventricular septum and the right ventricular free wall, as well as the left atrium were carefully separated, weighed and frozen. After 24 h, the infarcted area was identified on the basis of the pale color of the nonperfused region compared to the surrounding myocardium.

2.3. Preparation of RNA and Northern blot analysis

Total RNA isolation and Northern blot analysis were performed using standard protocols [15]. The densitometric value for each mRNA of interest was normalised for loading with the 18S rRNA signal.

Plasmids containing a full-length insert encoding the rat GLUT-1 and GLUT-4 proteins were generous gifts from Dr. B. Thorens (University of Lausanne, Switzerland). The cDNA encoding for the α-smooth muscle actin (α-SMA) was purchased from ATCC (Rockville, MA, USA). cDNA probes encoding for the following proteins were kindly provided by other research groups: 18S by Dr. R. Zimmermann (Max-Planck-Institute, Bad Nauheim, Germany), atrial natriuretic factor (ANF) by Dr. K.R. Chien (University of California, San Diego, CA, USA), long-chain acyl-CoA dehydrogenase (LCAD) and MCAD by Dr. D.P. Kelly (Washington University School of Medicine, St. Louis, MO, USA), very long-chain acyl-CoA dehydrogenase (VLCAD) by Dr. A.W. Strauss (Washington University School of Medicine), muscle carnitine palmitoyl transferase I ($CPT\text{-}1_M$) by Dr. J.D. McGarry (University of Texas Southwestern Medical Center, Dallas, TX, USA) and heart-fatty acid binding protein (H-FABP) by Dr. D.P. Cistola (Washington University School of Medicine).

2.4. Preparation of membrane proteins and Western blot analysis

For immunoblot analysis of GLUT-1 and GLUT-4, total myocardial membranes were prepared according to Garvey et al. [16]. Detection of GLUT-1 and GLUT-4 proteins

were performed as described previously [15]. For the determinations of MCAD, LCAD and VLCAD total cellular proteins were isolated and analyzed by immunoblotting using a standard protocol [17]. Blots detected by chemiluminescence (ECL, Amersham) were exposed to films. Quantitative analysis of bands on films was done by laser densitometry (*Imagequant 3.3, Molecular Dynamics).*

A polyclonal antibody directed against the 12-amino-acid carboxy-terminal sequence of rat brain GLUT-1 and a monoclonal 1F8 antibody directed against a GLUT-4 epitope were purchased from Biogenesis (UK). Polyclonal rat antibodies against VLCAD, LCAD and MCAD were kindly provided by Dr. A.W. Strauss (Washington University School of Medicine). Peroxidase-labeled polyclonal anti-rabbit and anti-mouse antibodies were purchased from Sigma.

2.5. Statistical analysis

All values are expressed as means±S.E.M. Optical densities of blots are expressed as percentage of the mean value of corresponding measurements in sham-operated hearts. Student's *t*-test for unpaired samples was used to compare values between infarcted and sham-operated hearts. Differences were considered significant at $P<0.05$.

3. Results

3.1. General characteristics of animals and hearts

Rats with infarction sacrificed after 24 h or 8 weeks exhibited less than three criteria of heart failure [14]. Twenty-four hours after infarct-induction, the heart weight to body weight (HW/BW) ratio was comparable between sham-operated rats and rats with infarction (Table 1). However, 8 weeks after surgery, HW/BW was significantly increased by 21% ($P<0.05$). In addition, the left atrium to body weight ratio (LA/BW) was increased by 150% ($P<0.01$).

Twenty weeks after surgery, six of sixteen rats (38%) with infarction exhibited all five criteria of heart failure, whereas the remaining ten animals exhibited less than three criteria.

The HW/BW and LA/BW ratios (Table 1) were higher in rats with heart failure than in rats without heart failure (+24% and +77%, respectively; $P<0.05$ for both comparisons) and than in sham-operated rats (+31% and +127%, respectively; $P<0.05$ for both comparisons). Furthermore, the ratio right ventricular free wall to body weight was significantly higher in rats with heart failure than in rats with infarction, but without heart failure (+105%; $P<0.05$) and in sham-operated rats (+108%; $P<0.05$).

3.2. Myocardial mRNA expression of ANF and α-SMA

ANF mRNA expression was increased in left ventricular myocardium as early as 24 h after infarction, in both the peri-infarction region (197±15%; $P<0.01$) and the septum (330±19%; $P<0.001$) compared to the corresponding mean values measured in sham-operated rats and only slightly increased further until 8 weeks (Fig. 1). Furthermore, at 8 weeks, average ANF mRNA was slightly, but significantly, increased in the right ventricular free wall (212±27%) compared to sham-operated rats ($P<0.05$). ANF expression in the left ventricular free wall and in the septum was further increased by 20 weeks in rats without and, to a larger extent, in rats with heart failure. At this time-point, ANF mRNA expression was increased in the right ventricular free wall only in rats with heart failure (492±160%; $P<0.05$) (Fig. 1). mRNA expression of α-SMA was increased 24 h after LAD ligation in the peri-infarction region (152±13%; $P<0.05$) compared to the

Table 1
Weight characteristics of sham-operated rats (sham) and rats with coronary ligation (infarcted)

	n	BW (g)	HW (g)	HW/BW (×1000)	LA/BW ($\times10^6$)	RV/BW (×1000)
Rats sacrificed after 24 h						
Sham	7	257±3	1.2±0.1	4.64±0.2	Nd	0.70±0.04
Infarcted	10	246±6	1.3±0.1	5.4±0.3	Nd	0.75±0.05
Rats sacrificed after 8 weeks						
Sham	9	456±25	1.8±0.1	3.9±0.2	99±28	0.59±0.09
Infarcted	10	462±10	2.1±0.1*	4.7±0.2*	248±40*	0.81±0.11
Rats sacrificed after 20 weeks						
Sham	8	500±23	1.7±0.1	3.5±0.2	96±9	0.56±0.06
Infarcted						
Without heart failure	10	509±15	1.9±0.15	3.7±0.2	123±12	0.57±0.05
With heart failure	6	535±34	2.4±0.2*	4.6±0.5*	218±33*	1.17±0.08*

Results are expressed as mean±S.E.M.; *n*, number of rats; HW, heart weight; BW, body weight; LA, left atrial weight; RV, weight of right ventricular free wall; nd, not determined.

*, $P<0.05$, vs. sham-operated rats.

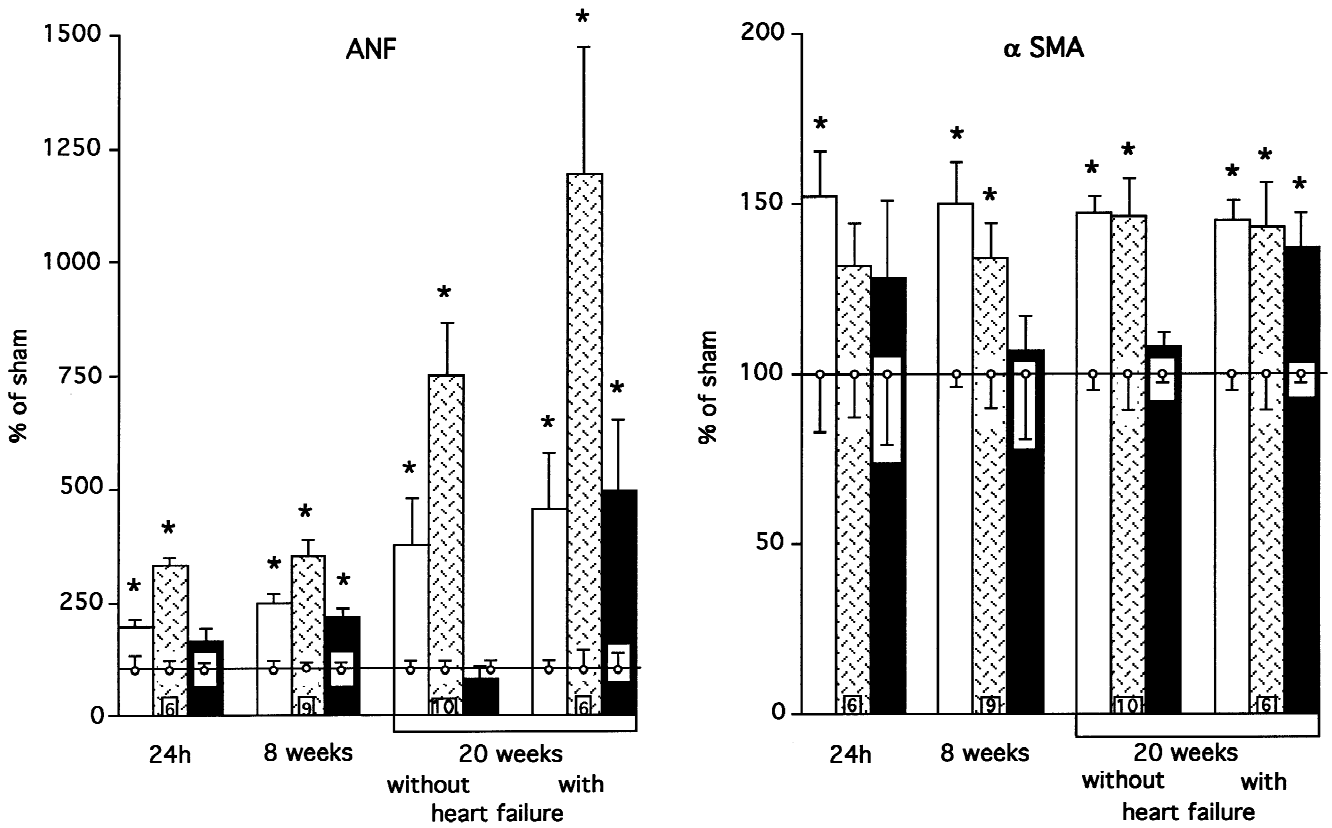

Fig. 1. Development of myocardial hypertrophy is associated with increased expression of mRNA encoding for the atrial natriuretic factor (ANF) and α-smooth muscle actin (α-SMA). The graph displays mRNA content in the peri-infarction region (□), the septum (▨) and the right ventricular free wall (■) 24 h, 8 and 20 weeks after coronary ligation. Photodensity values of ANF and α-SMA were corrected for loading using the 18S signal and expressed in % of the mean value of sham-operated rats. The S.E.M. of mean of values of optical densities measured in sham-operated rats and normalized to 100% are indicated (○). The number of infarcted animals for each time point is displayed in the septum bar. The number of sham-operated rats was at least six for each comparison. Results are expressed as mean±S.E.M. *, $P<0.05$ versus sham-operated hearts.

corresponding mean value of sham-operated rats (Fig. 1). Subsequently, α-SMA mRNA expression was also increased in the interventricular septum (8 and 20 weeks) and, after 20 weeks in the right ventricular free wall of rats with heart failure (137±10%; $P<0.05$) compared to sham-operated rats (Fig. 1).

3.3. Regional expression of GLUT-1 and GLUT-4

At 24 h after LAD ligation, GLUT-1 mRNA was increased in both the peri-infarction region (171±18%; $P=0.01$) and the septum (178±18%; $P=0.004$) compared to the corresponding values measured in sham-operated rats (Fig. 2). GLUT-4 mRNA tended to be lower in both regions (72±12 and 90±9%, respectively) compared to sham-operated rats, although statistical significance was achieved only for the peri-infarction region (Fig. 2). mRNA expression of GLUT-1 and GLUT-4 had returned towards baseline 8 weeks after infarction. At 20 weeks after infarct-induction, myocardial GLUT-1 mRNA content was increased again in both regions. The increase was much more pronounced in failing hearts averaging 172±28% ($P=0.001$) in the peri-infarction region and 230±40% ($P=0.001$) in the septum compared with the corresponding values measured in sham-operated rats (100±11 and 100±10%, respectively). In rats with heart failure, GLUT-4 mRNA was reduced in the peri-infarction region to 70±4% ($P=0.03$) and in the right ventricular free wall to 55±3% ($P<0.01$) compared to sham-operated rats, whereas no significant change of GLUT-4 mRNA was observed in rats without heart failure (Fig. 2).

Protein levels of GLUT-1 were increased 24 h after surgery to 162±9% in the peri-infarction region of infarcted hearts compared with 100±12% in sham-operated hearts ($P<0.05$) but did not differ any more significantly between infarcted and sham-operated hearts 8 weeks after surgery (93±3 and 100±7%; P=NS, respectively). Protein levels of GLUT-4 were slightly decreased 24 h after surgery to 82±8% in the peri-infarction region of infarcted hearts compared with sham-operated hearts (100±9%; P= NS) and were comparable in infarcted and sham-operated hearts 8 weeks after surgery (110±4 and 100±6%, respectively; P=NS).

Twenty weeks after infarction, myocardial GLUT-1 protein content was more than doubled in both the peri-infarction region (221±25%; $P=0.008$) and in the septum

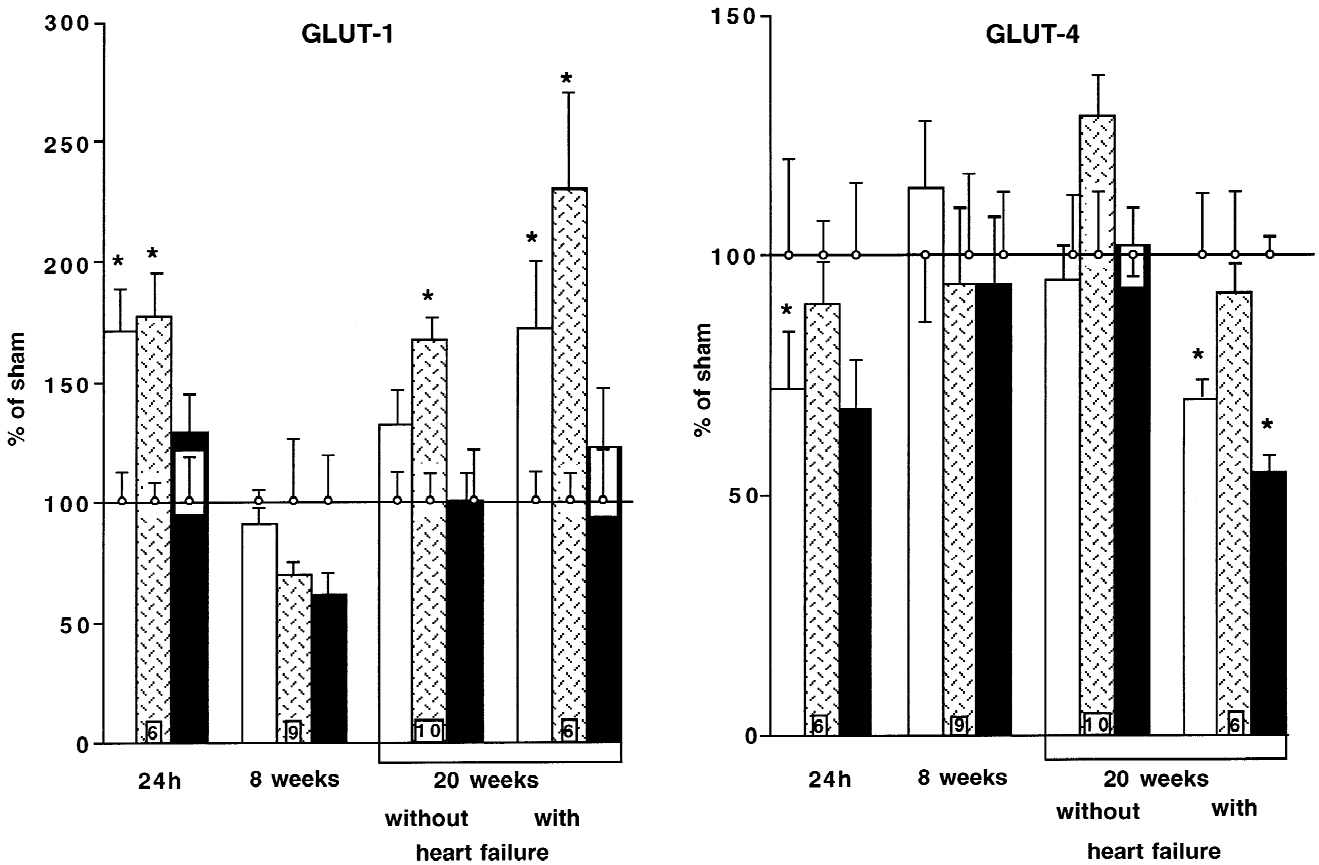

Fig. 2. Myocardial content of GLUT-1 mRNA (left) and GLUT-4 mRNA (right) in the peri-infarction region (□), the septum (▨) and the right ventricular free wall (■) 24 h, 8 and 20 weeks after surgery. Photodensity values of GLUT-1 and GLUT-4 were corrected for loading using the 18S signal and expressed as percentage of mean value of the sham-operated rats ($n=6$ at each time point). The S.E.M. of mean of values of optical densities measured in sham-operated rats and normalized to 100% are indicated (○). The number of infarcted hearts for each time point is indicated in the septum bar. Results are expressed as mean±S.E.M.; *, $P<0.05$ versus sham-operated hearts.

(212±11%; $P=0.002$) of rats with heart failure (Fig. 3). GLUT-4 protein content was decreased to 71±9% ($P=0.03$) in the peri-infarction region but not changed in the septum (90±4%; $P=$NS) of rats with heart failure compared to sham-operated rats (Fig. 3). In contrast to infarcted hearts with heart failure, no significant change of GLUT-1 and GLUT-4 protein content was observed in the peri-infarction region and the septum of infarcted hearts without heart failure. No change in GLUT-1 or GLUT-4 protein content was observed in the right ventricular free wall of rats with or without infarction.

3.4. Regional expression of genes encoding for regulatory proteins of fatty acid metabolism

At 24 h and 8 weeks after induction of infarction, myocardial mRNA expression of H-FABP and MCAD was significantly reduced in the peri-infarction region (Table 2). Mean protein level of MCAD was reduced, but statistically not different, after 24 h (−19±3%; $P=0.2$) and 8 weeks (−30±7%; $P=0.1$) in the peri-infarction region of infarcted rats compared to sham-operated rats (100±8 and 100±11%, respectively).

In contrast to markers of hypertrophy (ANF, α-SMA), no changes in mRNA and protein levels were observed for regulatory proteins of fatty acid metabolism in the septum and the right ventricular free wall 24 h and 8 weeks after surgery (data not shown).

Twenty weeks after coronary ligation, the expression pattern of regulatory proteins of fatty acid metabolism differed markedly between hearts with and without heart failure. In rats without heart failure, there were comparatively modest changes in mRNA contents which were statistically not significant (Table 2). mRNA and protein content of MCAD were reduced to 84±5% ($P=$NS) and 68±7% ($P<0.05$) compared to sham-operated rats in the peri-infarction region (Table 2, Fig. 4). Conversely, no change was observed for mRNA expression of H-FABP, CPT-1_M, LCAD and VLCAD (Table 2).

In rats with heart failure, myocardial mRNA and protein contents of MCAD were markedly decreased not only in the peri-infarction region but also in the septum and in the right ventricular free wall (Table 2, Figs. 4 and 5). Mean

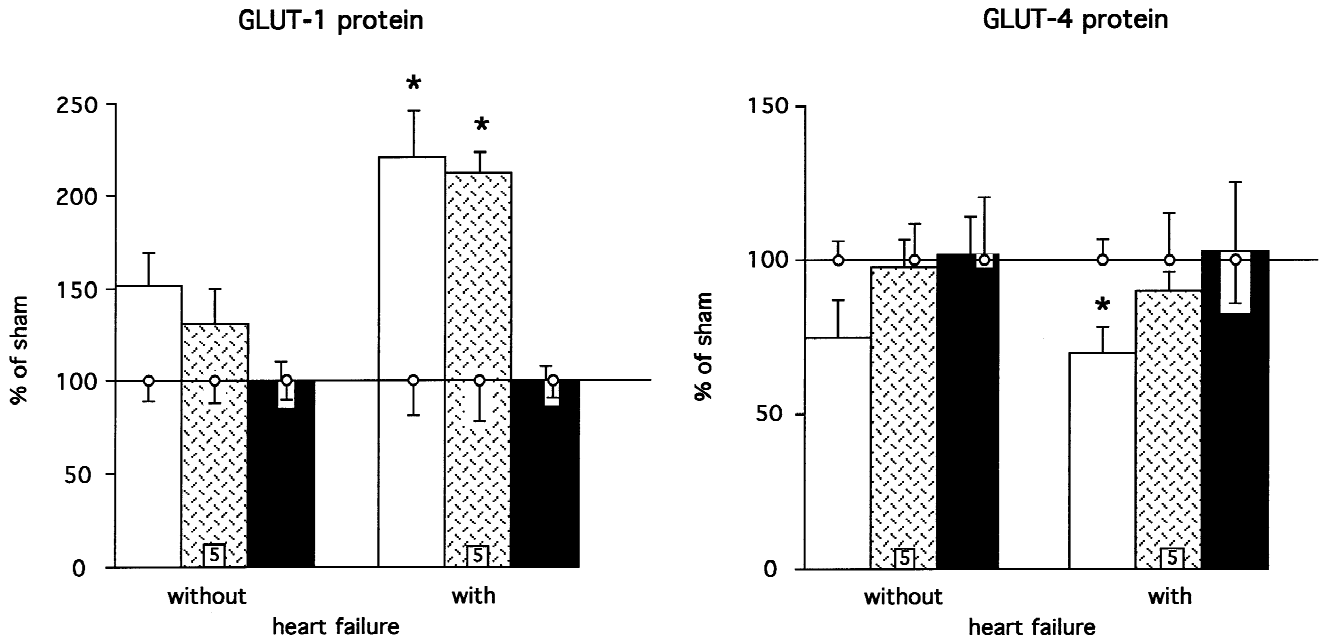

Fig. 3. Myocardial protein levels of GLUT-1 (left) and GLUT-4 (right) in the peri-infarction region (□), the septum (▨) and the right ventricular free wall (■) in rats without and with heart failure 20 weeks after surgery. Photodensity values are mean±S.E.M. and expressed as percentage of the mean value of sham-operated rats. The S.E.M. of mean of values of optical densities measured in sham-operated rats and normalized to 100% are indicated (○). The number of infarcted hearts is indicated in the septum bar. The number of sham-operated rats was at least four for each comparison. *, $P<0.05$ compared to the corresponding region in sham-operated hearts.

mRNA expressions of the other investigated acyl-CoA dehydrogenase enzymes of β-oxidation, LCAD and VLCAD, were also reduced in the left and right ventricular myocardium to a variable extent (Fig. 5). Reduction of protein content of LCAD and VLCAD was statistically not significant (94±4%; P=NS and 99±8%; P=NS, respectively, in the interventricular septum). The content of mRNA encoding for H-FABP was reduced in all regions, that of CPT-1_M in the septum and the right ventricular free wall (Fig. 5).

4. Discussion

Myocardial hypertrophy in response to hemodynamic overload of adult hearts is associated with a shift from fatty acid to glucose metabolism [5–9] and altered expression of regulatory proteins of energy metabolism [9,11,12]. The present study demonstrates that during postinfarction remodeling of the left ventricle, the expression of several developmentally regulated proteins involved in the control of glucose and fatty acid metabolism regresses to a ‘fetal’

Table 2
mRNA expression of genes involved in fatty acid metabolism in the peri-infarction region of rats 24 h, 8 or 20 weeks after coronary ligation or sham operation

	24 h		8 weeks		20 weeks		
	Infarcted (n=6)	Sham (n=6)	Infarcted (n=6)	Sham (n=6)	Without HF (n=10)	With HF (n=6)	Sham (n=6)
H-FABP	68±6*	100±6	77±4*	100±12	91±5	80±5*	100±9
CPT-1_M	96±6	100±7	96±9	100±21	107±11	98±5	100±16
MCAD	65±9*	100±10	75±7*	100±4	84±5	71±9*	100±8
LCAD	80±7	100±8	105±9	100±9	97±4	91±7	100±6
VLCAD	125±12	100±19	100±12	100±23	91±11	73±7*	100±12

HF, heart failure; H-FABP, heart-fatty acid binding protein, CPT-1_M, muscular carnitine palmitoyl transferase-1, MCAD, medium-chain acyl-CoA dehydrogenase, LCAD, long-chain acyl-CoA dehydrogenase, VLCAD, very-long chain acyl-CoA dehydrogenase. Values are percentage of mean value measured in sham-operated rats (n=6) and are expressed as means±S.E.M.

*, $P<0.05$ versus sham-operated rats.

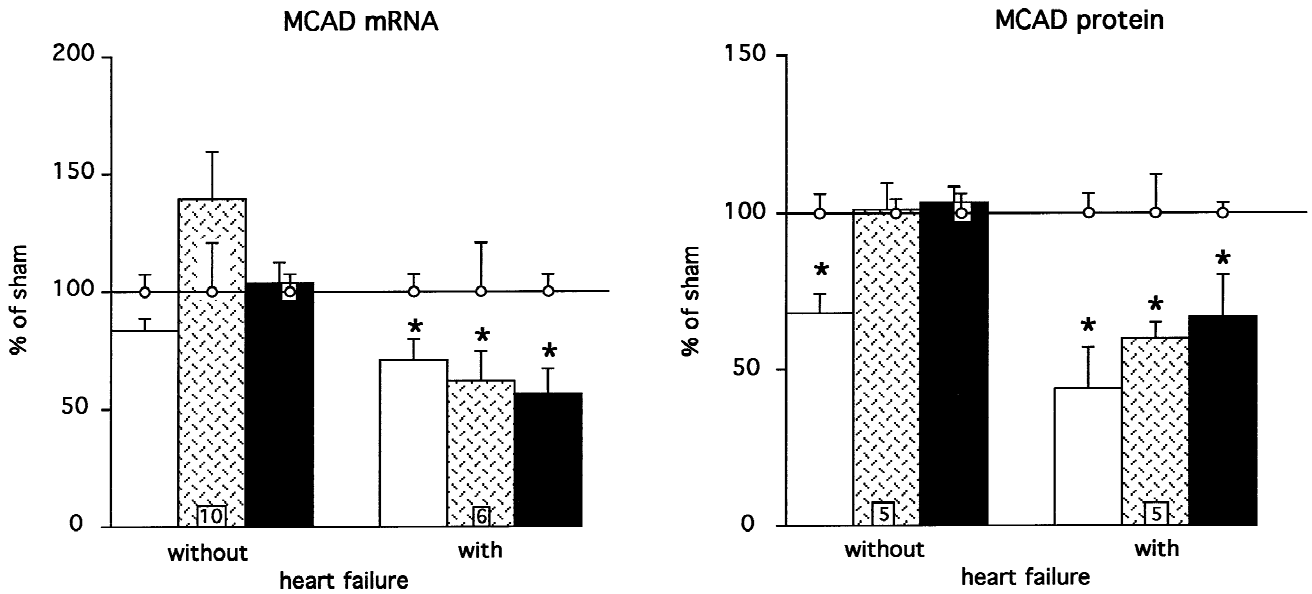

Fig. 4. Myocardial mRNA (left) and protein content (right) of medium-chain acyl-CoA dehydrogenase (MCAD) in rats 20 weeks after LAD ligation, in the peri-infarction region (□), the septum (▨) and the right ventricular free wall (■). Photodensity values are means±S.E.M. and expressed as percentage of the mean of corresponding values of sham-operated rats. The S.E.M. of mean of values of optical densities measured in sham-operated rats and normalized to 100% are indicated (○). The number of infarcted hearts is indicated in the septum bar. The number of sham-operated hearts was six for mRNA determination and four for protein determination. *, $P<0.05$ compared to the corresponding region in sham-operated hearts.

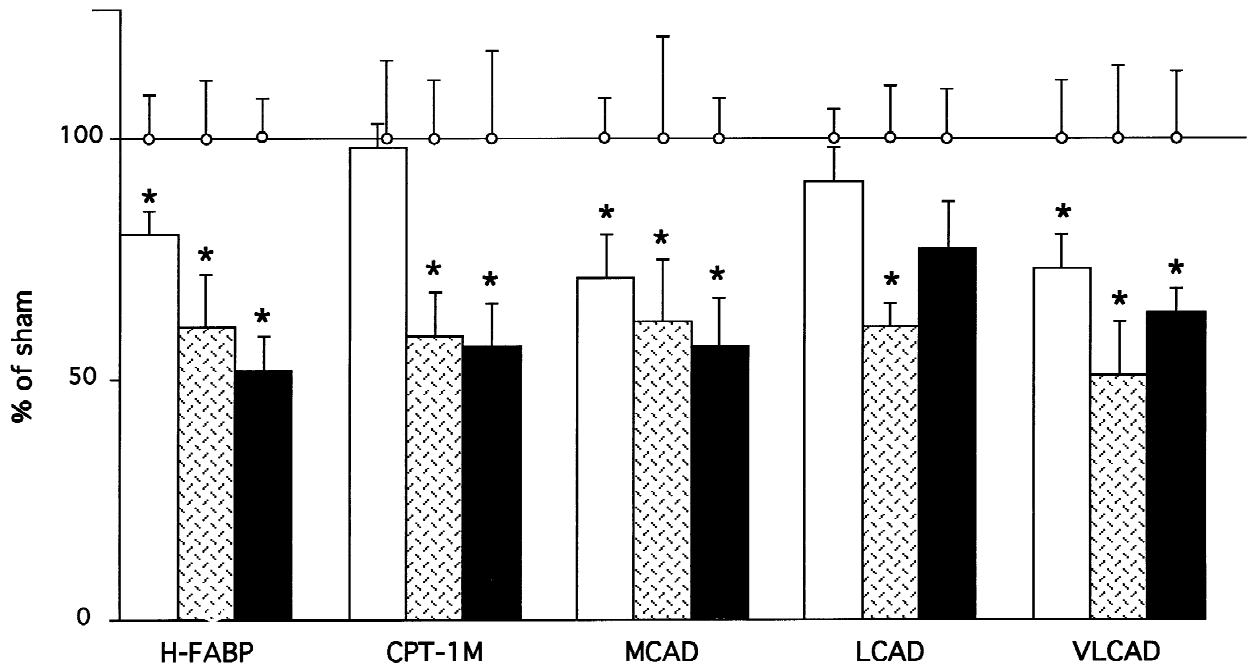

Fig. 5. Regional changes of myocardial mRNA content of heart-fatty acid binding protein (H-FABP), muscular carnitine palmitoyl transferase-1 isoform ($CPT\text{-}1_M$), medium-chain (MCAD), long-chain (LCAD) and very long-chain (VLCAD) acyl-CoA dehydrogenases 20 weeks after infarct-induction in rats with heart failure ($n=6$). mRNA contents are indicated for the peri-infarction region (□), the septum (▨) and the right ventricular free wall (■). Photodensity values were corrected for loading using the 18S signal and expressed as percentage of mean value of sham-operated rats ($n=6$). The S.E.M. of mean of values of optical densities measured in sham-operated rats and normalized to 100% are indicated (○). Values are means±S.E.M. *, $P<0.05$ compared to the corresponding region in sham-operated hearts.

type, favoring glucose metabolism. However, there exist pronounced differences in the time-course and regional distribution of observed changes in gene expression, which are influenced by the presence of heart failure.

In the present study the temporal and spatial heterogeneity of the expression of regulatory proteins of metabolism was studied in rats with large myocardial infarction comprising approximately 35% of the left ventricle [9]. Surviving myocardium exhibited hypertrophy based on unaltered or increased HW/BW, despite the presence of a large scar, and increased expression of ANF in the entire left ventricular myocardium. The observation period, in contrast to most published studies using this model, was extended to 20 weeks in order to examine changes in gene expression occurring during progression to heart failure, which developed between the 8th and 20th week in 38% of the rats with infarction. HW/BW after 20 weeks was higher in failing hearts than in non failing hearts, which, at least in part, is attributable to the increased weight of the right ventricular free wall. Increased weight and induction of ANF expression in the right ventricular wall of failing hearts is most likely the reflection of pulmonary hypertension. The lower HW/BW and LA/BW, as well as the absence of increased ANF mRNA in the right ventricular free wall, in the subgroup of rats without heart failure after 20 weeks, compared with rats examined 8 weeks after infarction, indirectly suggests that heart failure more frequently developed in rats with more pronounced left ventricular hypertrophy, higher left atrial pressure and right ventricular hypertrophy at 8 weeks. However, upon retrospective inspection of data, no relationship between HW/BW and alteration of gene expression was apparent after 8 weeks, which might have allowed identification of a subgroup of rats prone to develop heart failure.

The myocardium expresses two isoforms of glucose transporters, GLUT-1 and GLUT-4 [2]. The insulin-dependent isoform GLUT-4 predominates in adult myocardium, whereas the less insulin-sensitive isoform GLUT-1 predominates during prenatal life. GLUT-1 accounts for only approximately 20% of glucose transporters in adult myocardium [18]. In the present study, GLUT-1 mRNA expression was significantly increased in the entire left ventricular myocardium 24 h after coronary ligation. This observation corroborates the observation by Brosius et al. [19], of an increase of GLUT-1, mRNA and protein in remote myocardium as early as 6 h after coronary occlusion in dogs. However, increased GLUT-1 expression early after coronary occlusion seems to be transient, because we observed no significant difference between sham-operated and infarcted hearts after 8 weeks, when ANF was still increased. This suggests that increased GLUT-1 is neither a marker of hypertrophy nor responsible for increased glucose metabolism in hypertrophied myocardium. Consistent with this interpretation, Paternostro et al. [11] did not observe an increase of GLUT-1 mRNA in hypertrophied hearts from spontaneously hypertensive rats. In contrast to compensated left ventricular remodeling, in the present study, GLUT-1 mRNA and protein were approximately doubled in the entire left ventricular myocardium of rats exhibiting clinical signs of heart failure after 20 weeks.

The observed increase of GLUT-1 expression in the entire left ventricular myocardium early after coronary occlusion and after the occurrence of late onset heart failure suggests a relationship to the overall increase of wall stress and/or stress-related activation of humoral systems. The signaling pathway leading to enhancement of GLUT-1 is presently unknown. GLUT-1 expression was increased in response to angiotensin II in vascular smooth muscle cells [20] and to tumor necrosis factor α in L6 myotubes [21]. Preliminary results from our laboratory indicate that infusion of angiotensin II in rats in vivo markedly enhances expression of GLUT-1 [22]. Pronounced activation of the circulating renin–angiotensin system occurs at the onset of overt heart failure [23].

Similarly to GLUT-1, expression of GLUT-4 mRNA was not significantly modified during compensated remodeling 8 weeks and 20 weeks after coronary ligation, but was reduced early after infarct-induction and in failing left ventricles after 20 weeks. However, in contrast to the changes of GLUT-1, the reduction of GLUT-4 mRNA and protein was limited to the peri-infarction region. Although GLUT-1 and GLUT-4 often change concomitantly, but in the opposite direction, e.g. during postnatal development [2], hypoxia [24] and ischemia–reperfusion [15], the data of this study suggest that signaling pathways mediating altered expression differ for the two glucose transporter isoforms.

Sack et al. [12] have observed that the MCAD mRNA content was reduced in hypertrophied left ventricular myocardium in spontaneously hypertensive rats. Interestingly, the MCAD protein content was reduced only after the onset of overt heart failure [12]. The present study indicates that myocardial MCAD expression also decreases during postinfarction remodeling. However, reduced MCAD expression during postinfarction remodeling, without heart failure, differed from that observed during pressure overload in at least two respects. First, mRNA expression of MCAD was reduced only in the peri-infarction region without a significant change in the other regions participating in the hypertrophic response. Second, MCAD protein content was concomitantly reduced in this region even before the manifestation of overt heart failure. However, reduction of mRNA and protein expression of MCAD was detectable throughout the entire left ventricular myocardium and in the right ventricular free wall in rats with overt heart failure.

The signaling pathway inhibiting MCAD expression during postinfarction remodeling remains to be clarified. Leone et al. [25] have identified a nuclear receptor response element-1 (NRRE-1) in the promoter region of MCAD which activates transcription by binding a number of transcription factors, including peroxisome proliferator-

activated receptor α (PPARα). Recently Barger et al. [26] have provided evidence for inactivation of PPARα by phosphorylation-mediated by activation of the mitogen-activated protein kinases (MAPK) of the extracellular signal-regulated kinase family (ERK1/2) during myocardial hypertrophic growth. Because ERK1/2 are activated by mechanical stretch [27] it is conceivable that ERK1/2 activation, occurring initially in the peri-infarction region and spreading through the entire myocardium after decompensation, may contribute to reduced MCAD expression. At the level of transcription, Sack et al. [28] have observed increased Sp1 content in hypertrophied myocardium which may interfere with binding of PPARα to the NRRE-1.

Because, in addition to MCAD, activation of PPARα not only increases expression of MCAD but of a number of genes encoding proteins involved in fatty acid oxidation, including H-FABP [29], CPT-1_M [30], LCAD [31,32] and VLCAD [31,32], coordinate downregulation of mRNA expression of proteins involved in fatty acid metabolism might be expected during myocardial remodeling. Consistent with this hypothesis, reduction of myocardial mRNA of CPT-1_M [33] and LCAD [12] have been observed in animal models of myocardial overload. In the present study, the mRNA content of H-FABP, CPT-1_M, LCAD and VLCAD were reduced concomitantly with MCAD mRNA, at least in the septal region, in failing hearts after 20 weeks. However, there were considerable differences in the alteration of expression among proteins with respect to the time-course, the regional distribution and the association with onset of heart failure. This suggests that not a single mechanism, e.g. inactivation of PPARα, seems to be involved in altered expression of regulatory proteins of fatty acid metabolism during post-infarction remodeling of the left ventricle.

4.1. Clinical implications

It is presently unknown whether altered expression of regulatory proteins of substrate metabolism during post-infarction remodeling is an adaptative or maladaptative response, or simply an epiphenomenon. A shift from fatty acid to glucose metabolism may protect the myocardium by improvement of efficiency of oxidative metabolism (higher P/O-ratio), enhancement of glycolytic ATP-production [34] and prevention of apoptosis [35].

5. Study limitations

A number of study limitations need to be emphasized. First, the criteria of heart failure in rats proposed by Feldman et al. [14] are based on subjective appreciation. More prolonged observation (24 weeks) to achieve progression to heart failure in all rats, was abandoned because of unacceptably high mortality before sacrifice. However, advanced hemodynamic impairment, which is generally associated with cardiac decompensation, was also indicated by the higher left atrial weight, the presence of right ventricular hypertrophy and the increased expression of ANF in the right ventricular wall in the heart failure group. Second, the present study does not allow conclusions to be drawn on the metabolic consequences of observed modifications of gene expression. In particular, the results are not suitable to explain observations suggesting a shift from fatty acid to glucose oxidation in nonfailing hearts undergoing remodeling in response to infarction [9] or other conditions associated with myocardial overload [5–8]. Although the MCAD protein content was reduced by 30% after 8 weeks, the reduction did not achieve statistical significance, in contrast to an earlier study from our laboratory using a similar protocol [9]. Kantor et al. [36] have reported decreased activity of AMP-activated protein kinase during left ventricular volume overload, which may inhibit fatty acid oxidation by increased synthesis of malonyl-CoA, mediated by activation of the enzyme acetyl-CoA carboxylase. The relationship between altered gene expression and metabolic changes requires further clarification.

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